ACUTE AND CHRONIC EFFECTS OF THE INSECTICIDE ENDRIN ON RENAL FUNCTION AND RENAL HEMODYNAMICS

63-26

FEDERAL AVIATION AGENCY
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ABSTRACT

Chronic and acute effects of the insecticide endrin on renal function were studied in dogs. Animals were exposed to endrin chronically by intramuscular injection and acutely by intravenous infusion. In acute studies dogs developed systemic hypertension and increased renal vascular resistance attributable to a sympatho-adrenal action. Basic renal autoregulation was not impaired by endrin but was masked by effects of blood borne adrenergic agents. Changes in renal function were minimal. In chronic studies dogs developed progressive systemic hypotension with variable changes in renal function and terminal renal vasodilation in some instances. Pathological findings were minimal and could be related to hemodynamic alterations in the peripheral vasculature. Results from this investigation provide no evidence for renal failure due to chronic insecticide poisoning.

Biological effects of chlorinated hydrocarbon insecticides have been described (1, 2, 3). Endrin, the most toxic member of this group (2, 4) was chosen to be the subject of the present investigation. The effect of endrin on renal hemodynamics has not been described, but impairment of renal function has been noted in some cases of chlorinated hydrocarbon poisoning (5, 6, 7). Pathological changes in the kidney have been reported by Treon et al (2), although Conley (8) reports postmortem changes as nonspecific. The present studies were designed to determine if endrin has a direct action on renal hemodynamics and renal tubular function, as indicated by pressure flow relationships and changes in glomerular filtration rate, effective renal plasma flow, and tubular transport.

METHODS

Three types of experiments were carried out on adult mongrel dogs: acute endrin poisoning; chronic endrin poisoning; isolated perfused kidneys receiving blood from animals acutely poisoned with endrin.* Animals were anesthetized with sodium pentobarbital, 30 mg/Kg. Dogs in acute studies received a lethal dose of endrin (10 mg/Kg) in alcohol, while those in chronic experiments were given intramuscular injections of endrin in cotton seed oil (1 mg/Kg). Systemic arterial pressures were measured from cannulated femoral arteries with Statham pressure transducer connected to a Sanborn direct writing recorder.

*Endrin, obtained from Nutritional Biochemicals Corp., 21010 Miles Ave., Cleveland 28, Ohio.

A priming solution of mannitol, PAH, and creatinine was administered intravenously and blood levels were maintained by intravenous infusion. Dogs were infused until a urine flow of 1.5 to 2.0 ml/min was established.

1. ACUTE EXPERIMENTS:

A. Intact Kidney (in situ) Experiments: The left renal vein was catheterized for blood sampling. Four studies were carried out in which an alcoholic solution of endrin (25 mg/ml) was injected into the lumen of the small intestine. Eight additional animals were infused with endrin via the femoral vein. Endrin was administered after control clearance determinations. Clearance periods were carried out at half hour intervals for two to four hours. Blood pH and hematocrit determinations were also made.

Creatinine analysis was done by the method of Bosnes and Tausky (9), and PAH by the method of Smith et al (10). A Coleman flame photometer was used to determine sodium and potassium concentration of plasma and urine. Histological examinations were carried out on kidney, heart, lung, liver, spleen, adrenals, pancreas, brain and intestines.

B. Isolated Kidney Experiments: Six isolated kidney experiments were prepared as previously described (11) and perfused with heparinized blood from the femoral artery of anesthetized donor dogs. The kidney was transferred without interruption of blood flow and perfused at the systemic arterial pressure of the dog. Renal venous outflow was measured by a graduated cylinder and stop watch. Control flows averaged 3.4 ml/min/gm. The ureter was catheterized for urine flow measurement. Blood was returned to the femoral vein from a reservoir via a Sigma motor pump. Renal arterial pressure was monitored at the kidney inflow tubing. Convulsions were controlled by immobilizing the animal with succinylcholine chloride* (0.5 mg/Kg).

*Anectine, obtained from Burroughs Welcome Co., Tuckahoe, N.Y.

Respiration was maintained with a Starling respirator using room air. An alcoholic solution of endrin (25 mg/ml) was added to the blood reservoir at a rate of 0.5 ml/min.

The adrenergic blocking agent phentolamine**, which has little direct vascular effect

**Regitine, obtained from CIBA Pharmaceutical Co., Summit, N.J.

(12), was infused into the renal artery of the kidney before endrin as a control and 70-80 minutes post-endrin, to determine if changes in renal vascular resistance were due to blood borne adrenergic agents.

2. CHRONIC EXPERIMENTS:

Chronic studies were carried out on five female dogs, 14-20 Kg in weight. Two creatinine and PAH control clearance studies and tubular maximum (T_{max}) determinations were done on each animal. Injections of endrin were given five consecutive days each week to each dog. Weekly clearance studies were carried out during the post-endrin period. For each clearance determination the [illegible] artery was catheterized by a modification of the percutaneous method of Seldinger [illegible]. Renal vascular resistance (renal artery pressure/renal blood flow) was determined from data obtained from mean systemic arterial pressure, hematocrit, and PAH clearance. (The assumption was made that PAH extraction was complete and that the renal vein pressure remained low and constant).

Following sacrifice or death of the animals due to the effects of endrin, tissue samples were taken as described above.

RESULTS

1. ACUTE EXPERIMENTS:

A. Intact (in situ) Kidney Experiments (12 animals): The response of these animals to endrin was widely variable. Most of the measured parameters showed no consistent change. The experimental group in which endrin was injected into the lumen of the intestine did not develop convulsive signs of endrin poisoning during the two to four hour post-endrin period. Changes in renal vascular resistance in all animals were widely variable with a mean maximum change of +178% (range −28 to +900%). Glomerular filtration rate (C_{cr}) decreased a mean maximum of 46% (range −7 to −96%). PAH extraction ratio remained relatively constant. Urine flow was variable and urine concentration remained in the normal range (sp. gr. 1.010-1.030). Blood pH was constant and the hematocrit showed no consistent change (−13 to +8%).

The group receiving endrin intravenously developed convulsions within ten minutes after completion of the endrin infusion. Additional large quantities of sodium pentobarbital administered to suppress the convulsions caused blood pressure changes resulting in erratic kidney function. In order to avoid complications resulting from excessive pentobarbital administration, succinylcholine chloride was used to control convulsions in subsequent studies.

B. Isolated Kidney Experiments (6 kidneys): A marked increase in renal vascular resistance appeared in most kidneys within ten minutes of the time endrin infusion was completed. All kidneys showed an increased resistance within 60-70 minutes post-endrin (fig. 1). Phentolamine had very little effect on the renal vascular resistance when administered pre-endrin (fig. 1A), but caused a significant and sudden drop

in renal resistance when administered post-endrin (fig. 1B). The renal artery tubing was partially constricted in three experiments to lower renal artery pressure to control levels. In these animals injection of phentolamine caused the renal resistance to return to near control value. In the other animals the renal artery tubing was not constricted and injection of phentolamine caused a drop in renal resistance but to some level above control value. Dogs immobilized with succinylcholine chloride showed a consistent hypertension in response to endrin and occasionally an increased urine flow. Hypertension was also seen when convulsions appeared in the non-anectinized animal. No attempt was made to establish a diuresis in these kidneys and it was noticed that occasionally there was a complete cessation of urine flow. Addition of mannitol solution to the blood reservoir caused an almost immediate response by re-establishing a urine flow.

2. *Chronic Experiments (5 dogs):* The first noticeable sign of endrin poisoning was dysphagia. Muscle twitches of the tongue and throat made it difficult for the animals to eat. Most dogs developed hypotension within two weeks after initiation of endrin poisoning (table I). Glomerular filtration rates were variable (table II). A decrease in renal resistance was seen in some dogs (table III). Sodium and potassium absorption showed no consistent change (table V).

PATHOLOGICAL CHANGES:

Acute Studies: Renal tubular necrosis was seen in only one dog. There was a mild to moderate degree of protein precipitation in Bowman's space and in the tubules. An occasional glomerulus was observed in which a reflux of epithelium from the proximal convoluted tubule into Bowman's space had occurred.

The spleen exhibited moderate to severe congestion with early degenerative changes and a mild degree of necrosis in the reticular cells associated with the germinal centers. The degenerative changes were more prominent in the large active germinal centers. Mild to moderate congestion and irregularly distributed cloudy swellings were present in the liver. The lungs were slightly congested and contained occasional areas of hemorrhage, but the heart, pancreas, and intestine appeared normal.

Chronic Studies: No definite tubular changes were detected in the kidneys. The spleen exhibited a mild degree of inactivity of the germinal centers. There appeared to be some lipid depletion in the adrenal gland which showed mild to moderate congestion more pronounced near the cortico-medullary junction. In some dogs a moderate degree of passive congestion was seen in the liver. Mild congestion and occasional hemorrhage were found in the lungs of most animals. No significant pathological changes were seen in the brain, pancreas, lungs, heart, or intestine.

DISCUSSION

The constancy of the PAH extraction ratio in the acute studies of *in situ* kidneys suggests that endrin has no direct effect on the kidney in the first two to four hours after administration. Increased renal resistance and the effect demonstrated by phentolamine on the dog-pump perfused isolated kidney suggest action on the sympathoadrenal system. The increase in renal vascular resistance was not completely due to adrenergic agents since there was a small drop in renal vascular resistance when the renal artery pressure was reduced to control level in three kidneys. When phentolamine was administered, renal resistance returned to control levels. In other kidneys the artery was not clamped to reduce the pressure and the renal vascular resistance did not return completely to control level when phentolamine was administered but to some level above it. This difference from control values apparently represents the fraction of resistance change due to autoregulation within the kidney. The diuresis occasionally seen in acute endrin poisoning may be due to an increased resistance in the efferent arterioles. Administration of mannitol to re-establish urine flow in those animals with complete cessation of urine flow may indicate a form of treatment when this occurs in humans. The effect of mannitol in acute studies suggests that the infusion necessary for clearance studies constituted a form of treatment. It is a most significant observation that no evidence was obtained for renal failure due to chronic endrin poisoning.

Under the conditions of these experiments only mild pathological changes were seen in the kidney, liver, spleen, adrenal gland, and lungs. The appearance of protruded segments of proximal convoluted tubules is reported by Mayer and Ottolenghi (14) as occurring in apparently normal dogs. However, Wachstein and Meisel (15) and Waugh and Beschel (16) associated this phenomenon with ischemic damage to the kidneys. Reflux of tubular epithelium was observed occasionally in cases of acute toxicity but was apparently absent in chronically poisoned dogs. This is in agreement with the findings of Waugh and Beschel (16) with serotonin induced ischemia. Congestion of the liver, lungs, and adrenals did not appear to be of long duration and may have been associated with the terminal drop in blood pressure.

Dysphagia in chronic animals leading to starvation and dehydration appeared to account for systemic hypotension (17) which was reversed at first by infusion but in the moribund state became irreversible. There was no consistent change in renal function as a result of the development of hypotension (tables I, V).

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BIBLIOGRAPHY

1. T. E. Emerson, C. M. Brake and L. B. Hinshaw
CARI Report 63-16

2. J. F. Treon, and F. P. Cleveland
J. of Ag. and Food Chem. 3, 402, 1955

3. J. R. Busvine
Practitioner 188, 267, 1962

4. J. B. DeWitt
J. of Ag. and Food Chem. 3, 672, 1955

5. A. Bell
Med. J. of Australia 2, 698, 1960

6. E. J. Spiota
A. M. A. Arch. Ind. Hyg. 4, 500, 1951

7. E. Nelson
Rocky Mt. Med. J. 50, 483, June, 1953

8. B. E. Conley
J. A. M. A. 158:15, 1304, August 13, 1955

9. R. W. Bosnes and H. H. Tausky
J. Biol. Chem. 158, 581, 1945

10. W. W. Smith, N. Finkelstein and H. W. Smith
J. of Biol. Chem. 135, 231, August, 1940

11. L. B. Hinshaw, G. M. Bradley and C. H. Carlson
Am. J. Physiol. 196, 1127, 1959

12. H. D. Hohnson, H. D. Green and J. T. Lanier
J. of Pharmacol. and Exp. Ther. 108, 144 1953

13. S. I. Seldinger
Acta Radiologica 39, 368, January-June, 1953

14. E. Mayer and L. A. Ottolenghi
Anatom. Rec. 99, 177, 1947

15. M. Wachstein and E. Meisel
J. Histochem. and Cytochem. 5, 204, 1957

16. D. Waugh and H. Beschel
Amer. J. Path. 39, 547, 1961

17. C. M. Wilhelmj, A. J. Carnazzo and H. M. McCarthy
Am. J. of Phys. 191:1, 103, 1957

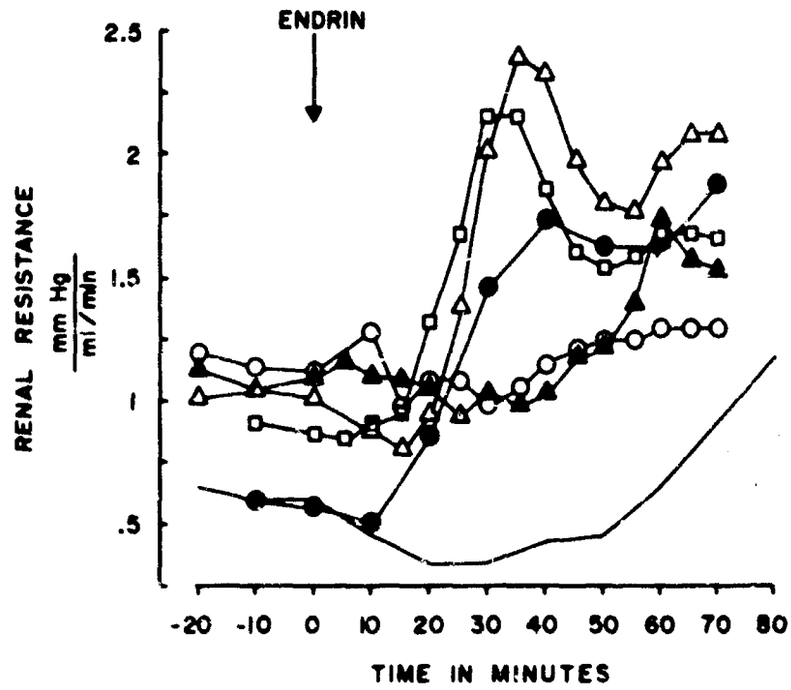

FIGURE 1

Figure 1 Changes in renal vascular resistance in isolated kidneys after administration of endrin

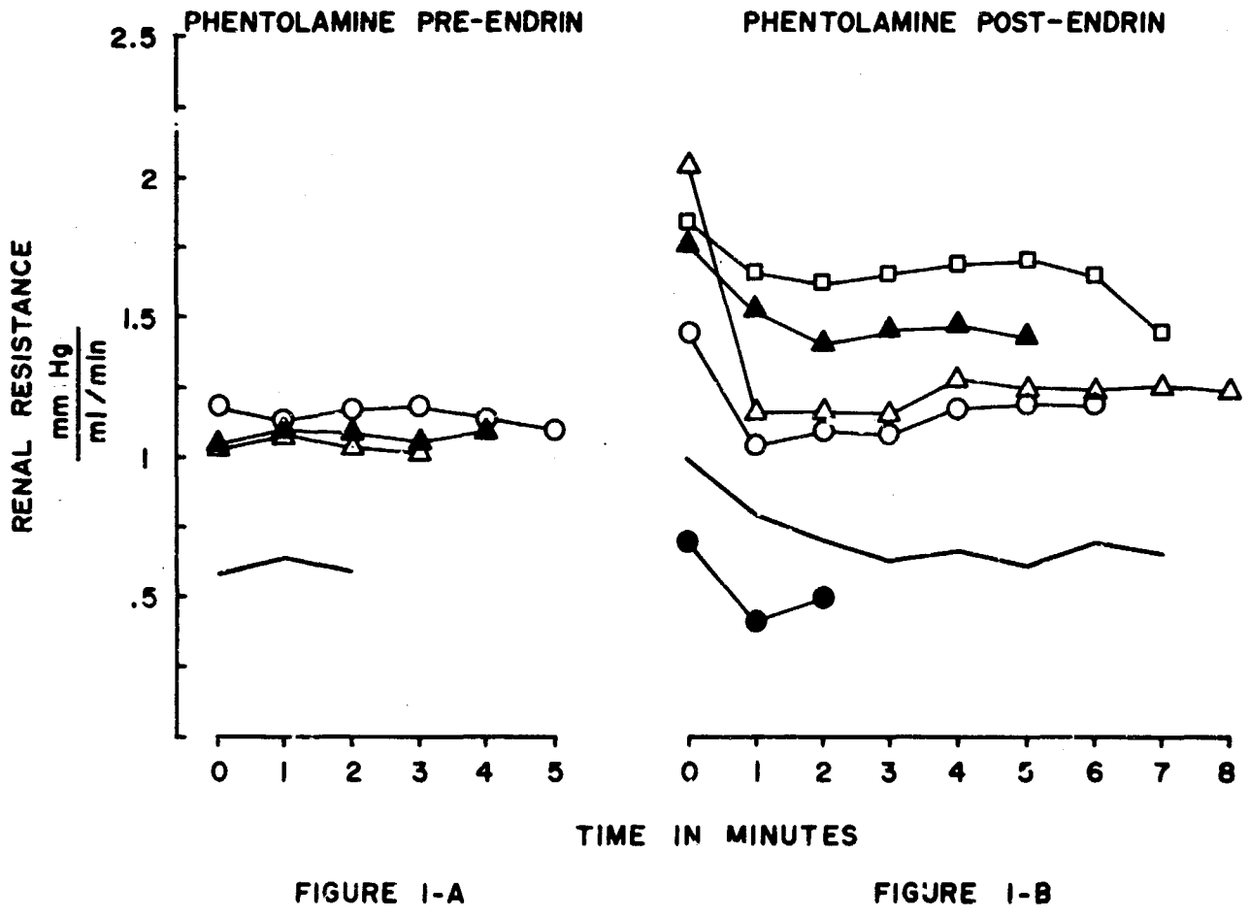

FIGURE 1-A

FIGURE 1-B

FIGURE 1A. Changes in renal vascular resistance in isolated kidneys with phentolamine only

FIGURE 1B. Changes in renal vascular resistance in isolated kidneys with phentolamine 70-80 min. after endrin.

TABLE I

Effect of Chronic Exposure to Endrin on Mean Systemic Arterial Pressure of Dogs

Dog	1 MSAP (mm Hg)		2 MSAP (mm Hg)		3 MSAP (mm Hg)		4 MSAP (mm Hg)		5 MSAP (mm Hg)	
Week	Pre* Infu.	Post Infu.	Pre Infu.	Post Infu.	Pre Infu.	Post Infu.	Pre Infu.	Post Infu.	Pre Infu.	Post Infu.
Control (2 Wk. Ave.)	135	142	105	118	105	138	110	110	80	106
1	145	149	130	124	90	130	95	120	75	105
2	115	134	80	125	125	126	90	125	85	101
3	90	122	80	101	105	151	85	111		
4	90	151	80	136	90	151	75	94		
5	105	136	90	126						
6	95	149	80	80						
7	95	131								
8	85	86								

*Pre Infusion = Value Prior to Infusion of Clearance Solution.

TABLE II

Effect of Chronic Exposure to Endrin on Glomeruler Filtration Rate in Dogs

Dog	1 GFR $\frac{(ml/min)}{(Kg)}$	2 GFR $\frac{(ml/min)}{(Kg)}$	3 GFR $\frac{(ml/min)}{(Kg)}$	4 GFR $\frac{(ml/min)}{(Kg)}$	5 GFR $\frac{(ml/min)}{(Kg)}$
Week					
Control (2 Wk. Ave.)	3.10	4.1	2.7	3.5	3.6
1	2.5	4.3	3.0	3.9	2.8
2	4.8	3.4	3.4	4.5	4.8
3	3.9	3.7	2.5	4.6	
4	3.2	3.8	3.0	2.2	
5	4.3	4.2			
6	3.5	4.0			
7	6.2				
8	6.4				

TABLE III

Effect of Chronic Exposure to Endrin on Renal Resistance and Clearance of PAH of Dogs

Dog	1		2		3		4		5	
Week	Renal Resist. (mm Hg)/(ml/min)	PAH (ml/min)/(Kg)	Renal Resist. (mm Hg)/(ml/min)	PAH (ml/min)/(Kg)	Renal Resist. (mm Hg)/(ml/min)	PAH (ml/min)/(Kg)	Renal Resist. (mm Hg)/(ml/min)	PAH (ml/min)/(Kg)	Renal Resist. (mm Hg)/(ml/min)	PAH (ml/min)/(Kg)
Control (2 Week Ave.)	0.6	8.28	0.3	15.8	0.3	11.78	0.4	11.82	0.7	8.42
1	0.7	6.67	0.4	11.44	0.4	10.47	0.4	15.16	1.0	6.20
2	0.4	12.21	0.5	10.33	0.2	15.00	0.3	15.79	0.6	11.78
3	0.4	11.56	0.3	9.90	..	.....	0.4	15.38		
4	0.5	9.85	0.4	11.74	0.4	11.91	0.4	16.65		
5	0.4	10.86	0.4	11.36						
6	0.5	10.05	0.2	13.33						
7	0.2	11.28								
8	0.2	15.24								

TABLE IV

Effect of Chronic Exposure to Endrin on Tubular Maximum Tm_{PAH} and Body Weight of Dogs

Dog	1			2			3			4			5	
Week	Tm PAH (mg/min)/(Kg)	Wt (Kg)	Hct (% RBC)	Tm PAH (mg/min)/(Kg)	Wt (Kg)	Hct (% RBC)	Tm PAH (mg/min)/(Kg)	Wt (Kg)	Hct (% RBC)	Tm PAH (mg/min)/(Kg)	Wt (Kg)	Hct (% RBC)	Tm PAH (mg/min)/(Kg)	Wt (Kg)	Hct (% RBC)
Control (2 Week Ave.)	0.54	18.6	40	0.55	18.6	36	.....	24.4	32	0.74	16.6	37	0.35	12.2	31
1	1.28	19.2	44	0.56	18.7	29	0.40	22.9	32	0.60	16.1	32	0.26	11.5	31
2	0.90	16.1	37	0.34	18	29	0.50	22.6	36	0.71	14.8	36	0.21	10	28
3	0.61	18.2	39	0.52	17.5	34	....	21.4	33	0.71	13.2	34			
4	0.55	17.3	41	0.44	16.8	34	0.79	19	37	0.41	11.6	30			
5	0.68	17.5	42	0.51	15.3	46									
6	0.67	17.0	47	0.25	14.4	41									
7	0.56	16.4	48												
8	0.31	15.7	46												

TABLE V

Effect of Chronic Exposure to Endrin on Sodium and Potassium Reabsorption of Dogs

Dog	1		2		3		4		5	
	Na (%) (reab.)	K (%) (reab.)	Na (%) (reab.)	K (%) (reab.)	Na (%) (reab.)	K (%) (reab.)	Na (%) (reab.)	K (%) (reab.)	Na (%) (reab.)	K (%) (reab.)
Week										
Control (2 Wk. Ave.)	98.6	93.8	96.8	78.1	.....	.....	96.8	92.8	96.1	87.0
1	99.5	93.1	96.9	83.0	98.9	91.8	97.1	86.9	97.1	94.2
2	99.2	88.8	96.4	86.2	96.0	82.8	97.8	91.5	98.3	96.8
3	99.4	91.6	95.2	90.5	98.9	88.0	97.4	95.4		
4	98.6	92.2	95.0	98.1	96.8	91.6	97.7	75.0		
5	99.0	94.0	96.8	89.6						
6	98.7	91.9	98.5	95.9						
7	98.8	81.4								
8	99.4	98.3								

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